



**TOXICOLOGY AND CARCINOGENESIS**

**STUDIES OF**

**METHYLPHENIDATE HYDROCHLORIDE**

**(CAS NO. 298-59-9)**

**IN F344/N RATS AND B6C3F<sub>1</sub> MICE**

**(FEED STUDIES)**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
**Public Health Service**  
**National Institutes of Health**

## FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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**NTP TECHNICAL REPORT**  
**ON THE**  
**TOXICOLOGY AND CARCINOGENESIS**  
**STUDIES OF**  
**METHYLPHENIDATE HYDROCHLORIDE**  
**(CAS NO. 298-59-9)**  
**IN F344/N RATS AND B6C3F<sub>1</sub> MICE**  
**(FEED STUDIES)**

**NATIONAL TOXICOLOGY PROGRAM**  
**P.O. Box 12233**  
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**Public Health Service**  
**National Institutes of Health**

## CONTRIBUTORS

### National Toxicology Program

*Evaluated and interpreted results and reported findings*

C.J. Alden, Ph.D.  
G.A. Boorman, D.V.M., Ph.D.  
D.A. Bridge, B.S.  
J.R. Bucher, Ph.D.  
J.K. Dunnick, Ph.D.  
S.L. Eustis, D.V.M., Ph.D.  
T.J. Goehl, Ph.D.  
J.R. Hailey, D.V.M.  
J.K. Haseman, Ph.D.  
G.N. Rao, D.V.M., Ph.D.  
J.H. Roycroft, Ph.D.  
B.A. Schwetz, D.V.M., Ph.D.  
D.B. Walters, Ph.D.  
K.L. Witt, M.S., Oak Ridge Associated Universities

### Hazleton Laboratories

*Conducted 14-day and 13-week studies,  
evaluated pathology findings*

K.M. MacKenzie, Ph.D., Principal Investigator  
B.H. Boysen, D.V.M., M.Sc.  
T.A. Jackson, D.V.M., Ph.D.

### TSI Mason Research Institute

*Conducted 2-year studies, evaluated pathology findings*

A.G. Braun, Sc.D., Principal Investigator  
A. Russfield, M.D.  
L.E. Sendelbach, Ph.D., D.A.B.T.  
F.A. Voelker, D.V.M., M.S., A.C.V.P.

### Experimental Pathology Laboratories, Inc.

*Provided pathology quality assurance*

J.F. Hardisty, D.V.M., Principal Investigator  
K. Yoshitomi, D.V.M., Ph.D.

### Dynamac Corporation

*Prepared quality assurance audits*

S. Brecher, Ph.D., Principal Investigator

### NTP Pathology Working Group

*Evaluated slides, prepared pathology report on rats  
(11 February 1992)*

D.G. Goodman, V.M.D., Chair  
PATHCO, Inc.  
T.A. Bertram, D.V.M., Ph.D.  
Proctor & Gamble, Co.  
S.L. Eustis, D.V.M., Ph.D.  
National Toxicology Program  
J.R. Hailey, D.V.M.  
National Toxicology Program  
R.A. Herbert, D.V.M., Ph.D.  
National Toxicology Program  
C.C. Shackelford, D.V.M., M.S., Ph.D.  
National Toxicology Program  
K. Takahashi, D.V.M., M.Sc., Ph.D.  
National Toxicology Program  
K. Yoshitomi, D.V.M., Ph.D.  
Experimental Pathology Laboratories, Inc.

*Evaluated slides, prepared pathology report on mice  
(17 March 1992)*

D.G. Goodman, V.M.D., Chair  
PATHCO, Inc.  
W.W. Carlton, D.V.M., Ph.D.  
Purdue University  
F. Chatani, Ph.D.  
Takeda Chemical Industries, Ltd.  
S.L. Eustis, D.V.M., Ph.D.  
National Toxicology Program  
J. Everitt, D.V.M.  
Chemical Industry Institute of Toxicology  
J.R. Hailey, D.V.M.  
National Toxicology Program  
R.A. Herbert, D.V.M., Ph.D.  
National Toxicology Program  
C.C. Shackelford, D.V.M., M.S., Ph.D.  
National Toxicology Program  
K. Yoshitomi, D.V.M., Ph.D.  
Experimental Pathology Laboratories, Inc.

### Biotechnical Services, Inc.

*Prepared Technical Report*

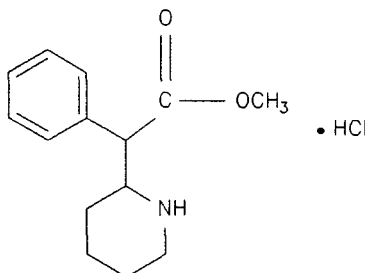
D.D. Lambright, Ph.D., Principal Investigator  
S.R. Gunnels, M.A.  
T.A. King-Hunter, B.S.  
H.A. Lindsay, B.A.

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## ABSTRACT



### METHYLPHENIDATE HYDROCHLORIDE

CAS No. 298-59-9

Chemical Formula:  $C_{14}H_{19}NO_2 \cdot HCl$

Molecular Weight: 269.77

**Synonyms:**  $\alpha$ -phenyl-2-piperidineacetic acid methyl ester hydrochloride; methylphenidylacetate hydrochloride;  
 $\alpha$ -phenyl- $\alpha$ -(2-piperidyl)acetic acid methyl ester hydrochloride; methyl  $\alpha$ -phenyl- $\alpha$ -(2-piperidyl)acetate hydrochloride  
**Trade names:** Centedrin; Centedrine; Ciba; Meridil; Phenidylate; Ritalin; Ritalin Hydrochloride

Methylphenidate hydrochloride is a drug used in the treatment of narcolepsy and attention deficit hyperactivity disorders. This drug was nominated for study by the Food and Drug Administration and the National Cancer Institute because of its widespread use in human medicine and because of lack of data on its potential carcinogenicity. Oral administration is the most common route of human exposure. Toxicology and carcinogenicity studies were conducted by administering methylphenidate hydrochloride (USP grade) *ad libitum* in feed to groups of male and female F344/N rats and B6C3F<sub>1</sub> mice for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in cultured Chinese hamster ovary cells.

#### 14-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were fed diets containing 0, 16, 62, 250, 1,000, or 4,000 ppm methylphenidate hydrochloride for 14 days. All rats survived to the end of the study. The final mean body weights of 4,000 ppm male and female rats were 9% lower than those of the con-

trols. Absolute and relative liver weights of 4,000 ppm males and females were significantly greater than those of the controls. Clinical findings during the first week of the study included hyperactivity in 4,000 ppm males and females, but these animals appeared to be normal during the second week of treatment. No treatment-related gross lesions were observed; however, centrilobular hypertrophy was observed in 4,000 ppm males and females.

#### 14-DAY STUDY IN MICE

Groups of five male and five female B6C3F<sub>1</sub> mice were fed diets containing 0, 16, 62, 250, 1,000, or 4,000 ppm methylphenidate hydrochloride for 14 days. Three 4,000 ppm males died during the second week of the study; all other mice survived to the end of the study. The final mean body weight of 4,000 ppm females was 11% lower than that of the controls, and the mean body weight gains of 1,000 and 4,000 ppm males and females were also significantly lower than those of the controls. Absolute and relative liver weights of all exposed groups of

males and of 4,000 ppm females were significantly greater than those of the controls. Hyperactivity was observed during the second week of the study in some 4,000 ppm males. Degeneration and necrosis of the renal tubule epithelium were observed in two 4,000 ppm males. Hepatocellular hypertrophy was observed in males and females exposed to 1,000 or 4,000 ppm and in males exposed to 250 ppm.

### 13-WEEK STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were fed diets containing 0, 125, 250, 500, 1,000, or 2,000 ppm methylphenidate hydrochloride for 13 weeks. There were no chemical-related effects on survival. Mean body weight gains of 500, 1,000, and 2,000 ppm males and females and of 250 ppm females were significantly lower than those of the controls. Final mean body weights of exposed males and females were similar to those of the controls. During the first week of the study, feed consumption by 2,000 ppm rats was less than that by controls, but during the remainder of the study feed consumption by exposed and control groups was similar. Rats exposed to 125, 250, 500, 1,000, or 2,000 ppm received approximate doses of 8, 15, 30, 70, or 130 mg methylphenidate hydrochloride per kilogram body weight per day (males) or 9, 18, 30, 70, or 150 mg/kg per day (females). Clinical findings in 1,000 and 2,000 ppm females included slight hypersensitivity to touch, hyperactivity, and increased vocalization during handling periods.

Absolute and relative liver weights of 2,000 ppm males and females were significantly greater than those of the controls, as were the relative liver weights of 1,000 ppm males and females. No chemical-related differences in bone length, bone density, or nose-to-rump lengths were noted in males or females, nor were there treatment-related histopathologic lesions.

### 13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F<sub>1</sub> mice were fed diets containing 0, 125, 250, 500, 1,000, or 2,000 ppm methylphenidate hydrochloride for 13 weeks. There were no chemical-related effects on survival. Final mean body weights of males exposed to 250, 500, 1,000, or 2,000 ppm and of 2,000 ppm females were significantly lower than those of the controls. The final mean body weights of other exposed male and female groups were similar to those of the controls. During the first week of the

study, feed consumption by 2,000 ppm mice was less than that by controls; feed consumption by exposed groups was similar to that by the controls throughout the remainder of the study. Mice exposed to 125, 250, 500, 1,000, or 2,000 ppm received approximate doses of 15, 30, 70, 115, or 230 mg/kg per day (males) or 15, 30, 70, 125, or 260 mg/kg per day (females). No chemical-related clinical findings were observed.

Absolute and relative liver weights of 1,000 and 2,000 ppm males and females were significantly greater than those of the controls, as were the relative liver weights of 125, 250, and 500 ppm males. Centrilobular hypertrophy and hepatocellular degeneration or necrosis were observed in males exposed to 500, 1,000, or 2,000 ppm methylphenidate hydrochloride.

### 2-YEAR STUDY IN RATS

Based on the increased liver weights and lower body weight gains in 2,000 ppm rats in the 13-week study, the high dose selected for the 2-year rat study was 1,000 ppm. Groups of 70 male and 70 female F344/N rats were fed diets containing 0, 100, 500, or 1,000 ppm methylphenidate hydrochloride for up to 2 years. As many as 10 male and 10 female rats per exposure group were evaluated at 9 or 15 months.

#### *Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings*

Survival of exposed rats was similar to that of the controls at the end of the study. Mean body weights of 500 and 1,000 ppm males were 3% to 10% lower than those of the controls from week 30 to the end of the study; during the same time period, mean body weights of 500 and 1,000 ppm females were 4% to 24% less than those of the controls. Final mean body weights of rats exposed to 100, 500, or 1,000 ppm were 102%, 95%, or 90% (males) and 96%, 89%, or 78% (females) those of the controls. Rats exposed to 100, 500, or 1,000 ppm methylphenidate hydrochloride in feed received approximate doses of 5, 25, or 50 mg/kg per day (males and females). The only chemical-related clinical finding was an increased incidence of fighting among group-housed males exposed to 1,000 ppm.

#### *Hematology and Clinical Chemistry*

No biologically significant differences in hematology or clinical chemistry parameters occurred at 9 or 15 months.

### **Pathology Findings**

In female rats exposed to 500 or 1,000 ppm, the incidence of mammary gland fibroadenomas was decreased (0 ppm, 15/49; 100 ppm, 13/50; 500 ppm, 6/48; 1,000 ppm, 5/50), and the decrease was considered to be related to chemical administration. No significant chemical-related increases in neoplasm incidences were observed in male or female rats.

### **2-YEAR STUDY IN MICE**

Based on the liver toxicity and lower body weight gains observed in 1,000 and 2,000 ppm mice in the 13-week study, the high dose selected for the 2-year study was 500 ppm. Groups of 70 male and 70 female B6C3F<sub>1</sub> mice were fed diets containing 0, 50, 250, or 500 ppm methylphenidate hydrochloride for 2 years. As many as 10 male and 10 female mice per exposure group were evaluated at 9 or 15 months.

#### ***Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings***

Survival of exposed mice was similar to that of the controls at the end of the study. Mean body weights of mice exposed to 250 or 500 ppm were 3% to 11% lower than those of the controls throughout much of the study; during the same time period, mean body weights of 250 ppm females were 3% to 7% lower than those of the controls. Final mean body weights of mice exposed to 50, 250, or 500 ppm were 97%, 89%, or 93% (males) and 98%, 93%, or 97% (females) that of the controls. Mice exposed to 50, 250, or 500 ppm methylphenidate hydrochloride in feed were estimated to have received 6, 30, or 60 mg/kg body weight per day (males) or 8, 40, or 80 mg/kg per day (females). There were no chemical-related clinical findings.

#### ***Hematology and Clinical Chemistry***

No biologically significant differences in hematology or clinical chemistry parameters occurred at 9 or 15 months.

### **Pathology Findings**

The principal lesions associated with the administration of methylphenidate hydrochloride occurred in the liver. A few hepatocellular neoplasms were

observed in control and exposed male mice at the 9- and 15-month interim evaluations, but the incidences in exposed groups were not significantly increased. At the end of the 2-year study, incidences of eosinophilic foci were increased in 500 ppm males and females. Increased incidences of hepatoblastoma occurred in 500 ppm males (0 ppm, 0/50; 50 ppm, 1/50; 250 ppm, 1/50; 500 ppm, 5/50). Increased incidences of hepatocellular adenoma also occurred in 500 ppm males (18/50, 18/50, 16/50, 29/50) and females (6/49, 10/48, 10/49, 28/50). The incidences of hepatocellular carcinoma were similar among control and exposed mice.

### **GENETIC TOXICOLOGY**

Methylphenidate hydrochloride was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation (S9). Methylphenidate hydrochloride was also tested for induction of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. In the chromosomal aberrations tests, positive results were not consistently dependent upon the presence or absence of S9 activation. Sister chromatid exchanges were not increased in the presence of S9, but one laboratory did obtain a positive response without S9 by testing higher doses than were used in tests with S9.

### **CONCLUSIONS**

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity\** of methylphenidate hydrochloride in male or female F344/N rats receiving 100, 500, or 1,000 ppm. There was *some evidence of carcinogenic activity* of methylphenidate hydrochloride in male and female B6C3F<sub>1</sub> mice based on the occurrence of hepatocellular neoplasms.

Treatment of female rats with methylphenidate hydrochloride was associated with a decrease in the incidence of mammary gland fibroadenomas. Administration of methylphenidate hydrochloride to male and female mice resulted in increased incidences of eosinophilic foci.

\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Report Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

### Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Methylphenidate Hydrochloride

	Male F344/N Rats	Female F344/N Rats	Male B6C3F <sub>1</sub> Mice	Female B6C3F <sub>1</sub> Mice
<b>Doses</b>	0, 100, 500, or 1,000 ppm in feed [approximately 5, 25, or 50 mg/kg/day]	0, 100, 500, or 1,000 ppm in feed [approximately 5, 25, or 50 mg/kg/day]	0, 50, 250, or 500 ppm in feed [approximately 6, 30, or 60 mg/kg/day]	0, 50, 250, or 500 ppm in feed [approximately 8, 40, or 80 mg/kg/day]
<b>Final mean body weights</b>	500 and 1,000 ppm groups slightly lower than controls	500 and 1,000 ppm groups lower than controls	250 ppm group lower than controls	Exposed groups similar to controls
<b>2-Year survival rates</b>	28/50, 33/50, 34/50, 34/51	31/50, 32/50, 36/50, 39/50	45/50, 45/50, 44/50, 41/50	37/50, 35/50, 37/50, 44/50
<b>Nonneoplastic effects</b>	None	None	<u>Eosinophilic foci:</u> 6/50, 8/50, 9/50, 14/50	<u>Eosinophilic foci:</u> 3/49, 3/48, 8/49, 25/50
<b>Neoplastic effects</b>	None	None	<u>Liver:</u> Hepatocellular adenoma: 18/50, 18/50, 16/50, 29/50; hepatoblastoma: 0/50, 1/50, 1/50, 5/50; hepatocellular adenoma, carcinoma, or hepatoblastoma: 24/50, 23/50, 26/50, 34/50	<u>Liver:</u> Hepatocellular adenoma: 6/49, 10/48, 10/49, 28/50; hepatocellular adenoma or carcinoma: 9/49, 11/48, 11/49, 30/50
<b>Decreased incidences</b>	None	<u>Mammary gland:</u> fibroadenomas: 15/49, 13/50, 6/48, 5/50	None	None
<b>Level of evidence of carcinogenic activity</b>	No evidence	No evidence	Some evidence	Some evidence
<b>Genetic toxicology</b>				
<i>Salmonella typhimurium</i> gene mutation: Negative in strains TA97, TA98, TA100, TA1535, and TA1537 with and without S9				
Sister chromatid exchanges				
Chinese hamster ovary cells <i>in vitro</i> : Positive without S9; negative with S9				
Chromosomal aberrations				
Chinese hamster ovary cells <i>in vitro</i> : Positive without S9 at first lab, positive with S9 at second lab				

## EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

**NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS  
TECHNICAL REPORTS REVIEW SUBCOMMITTEE**

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on methylphenidate hydrochloride on June 22, 1993, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

**Curtis D. Klaassen, Ph.D., Chair**  
Department of Pharmacology and Toxicology  
University of Kansas Medical Center  
Kansas City, KS

**Paul T. Bailey, Ph.D.**  
Environmental and Health Sciences Laboratory  
Mobil Oil Corporation  
Princeton, NJ

**Louis S. Beliczky, M.S., M.P.H.\***  
Department of Industrial Hygiene  
United Rubber Workers International Union  
Akron, OH

**Arnold L. Brown, M.D.**  
University of Wisconsin Medical School  
Madison, WI

**Kowetha A. Davidson, Ph.D.**  
Health and Safety Research Division  
Oak Ridge National Laboratory  
Oak Ridge, TN

**Harold Davis, D.V.M., Ph.D., Principal Reviewer**  
Medical Research Division  
American Cyanamid  
Pearl River, NY

**Daniel S. Longnecker, M.D.\***  
Department of Pathology  
Dartmouth Medical School  
Lebanon, NH

**Louise Ryan, Ph.D., Principal Reviewer**  
Division of Biostatistics  
Harvard School of Public Health and  
Dana-Farber Cancer Institute  
Boston, MA

**Ellen K. Silbergeld, Ph.D.\***  
University of Maryland Medical School  
Baltimore, MD

**Robert E. Taylor, M.D., Ph.D., Principal Reviewer**  
Department of Pharmacology  
Howard University College of Medicine  
Washington, D.C.

**Matthew J. van Zwieten, D.V.M., Ph.D.**  
Department of Safety Assessment  
Merck Research Laboratories  
West Point, PA

**Jerrold M. Ward, D.V.M., Ph.D.**  
National Cancer Institute  
Frederick, MD

**Lauren Zeise, Ph.D.**  
Reproductive and Cancer Hazard Assessment Section  
California Environmental Protection Agency  
Berkeley, CA

---

\* Did not attend

## SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 22, 1993, the draft Technical Report on the toxicology and carcinogenesis studies of methylphenidate hydrochloride received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of methylphenidate hydrochloride by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on chemical-related neoplastic and nonneoplastic lesions in mice. The proposed conclusions were: *no evidence of carcinogenic activity* in F344/N rats and *some evidence of carcinogenic activity* in B6C3F<sub>1</sub> mice based on the occurrence of hepatocellular adenomas.

Dr. Taylor, a principal reviewer, agreed with the proposed conclusions. He thought the discussion of metabolism and certain selective aspects of the stereochemistry related to metabolism was quite good. He found the genetic toxicology data hard to interpret.

Dr. Ryan, the second principal reviewer, agreed in principle with the proposed conclusions. She requested more detail on the trends for increased thyroid neoplasms because of the perceived hormonal effects of the chemical. Dr. Dunnick said the numbers didn't support calling this effect chemical related. Dr. Ryan thought there needed to be more discussion on whether the level of evidence in mice based on hepatocellular neoplasms should be raised. Dr. Ryan said that, because this drug is taken by young children, she was concerned that the animals were too old at study start and that bone density measurements might have been useful. Dr. Dunnick responded that the animals were six to seven weeks old at study initiation and that measurements taken during the study showed no effects on bone density. She noted that the purpose of this study was to assess the carcinogenic potential of methylphenidate hydrochloride and that ongoing studies of its other effects are being conducted by the National Institutes of Health.

Dr. Davis, the third principal reviewer, did not agree with the proposed conclusions for mice. He said a conclusion of *clear evidence of carcinogenic activity* is supported by dose-related increases in the incidence of hepatocellular adenoma and carcinoma (combined) and in the incidence of hepatoblastoma, a very rare and malignant neoplasm. Dr. Davis commented that the genetic toxicology section was too much a litany of results without a unifying conclusion regarding the genetic toxicology of the chemical. Dr. Dunnick explained that, in this study, the five *Salmonella* strains assayed were all negative, while some other genetic toxicology assays were positive. Dr. E. Zeiger, NIEHS, said that no generally accepted agreement on what defines genotoxicity in a chemical exists. He added that a revised write-up would be included in the report.

In response to the reviewers' concerns about the level of evidence in mice, Dr. J.R. Hailey, NIEHS, led a discussion about the nature of the hepatoblastomas. He said that, although little is known about this neoplasm, a few are being seen in mice from studies that do not yet appear in the NTP historical control database. Hepatoblastomas appear late in mice and are generally observed within other hepatocellular neoplasms, usually carcinomas, and may be considered a more primitive variant. He said the most appropriate treatment for statistical analysis of the hepatoblastomas should be to combine them with adenomas and carcinomas. Dr. Davidson asked that some of this discussion be summarized in the report. Dr. Ward also thought that the high incidence of hepatocellular neoplasms in females and the occurrence of rare neoplasms in males supported raising the conclusion to *clear evidence of carcinogenic activity* in mice. Dr. J.K. Haseman, NIEHS, defended the proposed conclusion, *some evidence*, because most of the increased neoplasms in exposed animals were benign and because all of the hepatoblastomas occurred in animals with other hepatocellular neoplasms, which did not increase the combined incidence.

Dr. Brown moved that the Technical Report on methylphenidate hydrochloride be accepted with the revisions discussed and with the conclusions as written. Dr. Taylor seconded the motion, noting that

the wording at the end of the first paragraph of the conclusions be changed from "adenomas" to "neoplasms." Dr. Zeise offered an amendment that the conclusion for male mice be changed to *clear evidence of carcinogenic activity*. Dr. Ward seconded the amendment, which was defeated by four no votes

(Drs. Bailey, Brown, Davidson, and Taylor) to three yes votes (Drs. Davis, Ward, and Zeise) with two abstentions (Drs. Ryan and van Zwieten). The original motion by Dr. Brown, including the wording change, was then accepted by eight yes votes with one abstention (Dr. van Zwieten).